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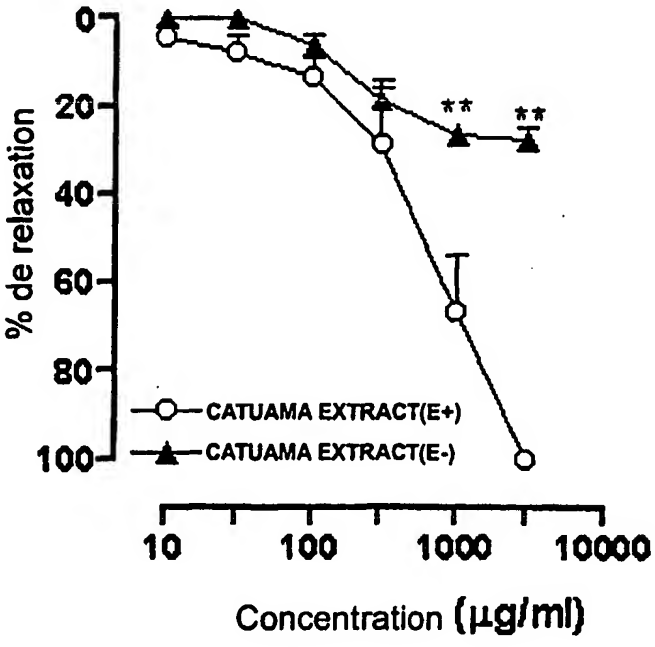
(54) Title: USE OF A PRODUCT COMPRISING CATUAMA EXTRACT AS AN ANTIOXIDANT AND CEREBRAL VASODILATOR AGENT



(57) Abstract: This invention relates to the use of a product comprising plant extracts comprising the species *Trichilia sp.*, *Paullinia cupana* (Sapindaceas), *Ptychopetalum olacoides* (Olacaceae) and *Zingiberaceae officinale* (*Zingiberaceae*), wherein said product is an antioxidant and/or cerebral vasodilator agent. A product particularly encompassed by the scope of the invention is Catuama extract commerically available as Catuama [R].

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Title:"**USE OF A PRODUCT COMPRISING CATUAMA EXTRACT AS AN ANTIOXIDANT AND CEREBRAL VASODILATOR AGENT, PHARMACEUTICAL COMPOSITION COMPRISING SUCH PRODUCT FOR PROPHYLAXIS OR TREATMENT OF VASCULAR DYSFUNCTIONS AND DISORDERS CAUSED BY THE IMPROPER PRESENCE OF FREE RADICALS, METHOD FOR THE PROPHYLAXIS OR TREATMENT OF CEREBRAL VASCULAR DYSFUNCTIONS AND DISORDERS CAUSED BY THE IMPROPER PRESENCE OF FREE RADICALS USING SAID PRODUCT AND USE OF SAID PRODUCT FOR MANUFACTURING A PHARMACEUTICAL COMPOSITION FOR THE PROPHYLAXIS OR TREATMENT OF VASCULAR DYSFUNCTIONS AND DISORDERS CAUSED BY THE IMPROPER PRESENCE OF FREE RADICALS".**

Field of the Invention

The present invention relates to the use of a product comprising Catuama extract, comprising species of *Trichilia sp.*, particularly *Trichilia catigua* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*).

Background of the Invention

Medicinal plants known as catuaba (Trichilia sp.) have recognized uses due to their aphrodisiac activities, as a tonic and in the treatment of physical and mental fatigue.

Already known are, e.g., phytotherapeutic formulations prepared from extracts of catuaba plants, which can be used alone or in combination with other medicinal plant extracts, such as guarana. A number of alternative formulations containing extracts of other species of catuaba are already well-known from the state-of-the-art, all of them being related to the tonic and stimulating effect of this group of plants.

There also exists in the art phytotherapeutic products comprising a combination of extracts of plants from the *Trichilia sp.* species, particularly *Trichilia catigua* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*).

A commercially available product comprising extracts of the above-mentioned plants in combination with suitable carriers is Catuama®. More particularly, Catuama® is a phytotherapeutical widely used in Brazil. Its composition consists of 4 extracts from medicinal plants including: catuaba (Trichilia catigua, A. juss, Meliaceae - (husk)), guarana (Paullinia cupana, K., Sapinadaceae - (seed)), muirapuama (Ptychopetalum olacoides, B., Olacaceae - (root)) and ginger (Zingiber officinale, L., Zingiberaceae - (rhizome)).

Summary of the Invention

The present invention refers to the use of a product of the extract of Catuama comprising *Trichilia sp.*, particularly *Trichilia catigua* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*) as an antioxidant or cerebral vasodilator agent.

In another aspect, this invention refers to pharmaceutical compositions comprising said extracts having antioxidant and cerebral vasodilator activities.

In yet another aspect, this invention refers to a method for the prophylaxis or treatment of cerebral vascular dysfunctions using said extract of Catuama.

In still another embodiment, the invention refers to the use of a product comprising extract of Trichilia sp., particularly Trichilia catigua (Meliaceae), Paullinia cupana (Sapindaceae), Ptychopetalum olacoides (Olacaceae) and Zingiber officinale (Zingiberaceae), for preparing a pharmaceutical composition for the prophylaxis or treatment of cerebral vascular dysfunctions.

Detailed Description of the Invention

After extensive studies, the inventors have found that the extract of Catuama, comprising *Trichilia sp.*, particularly *Trichilia catigua* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*), has extraordinary antioxidant and cerebral vasodilator activities.

As used herein "antioxidant activities or activities for prophylaxis

or treatment of cerebral vascular dysfunctions" includes activities related to disorders such as: loss of memory, vascular alterations, ischemia, difficulty in movements, difficulty in learning and concentrating, and for neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, which are pathologies associated, at least part, with prolonged exposure to the improper presence or excess of free radicals.

Examples of dysfunctions and uses according to the present invention comprise the prophylaxis and treatment of cerebral vascular dysfunctions, arteriosclerosis, stroke (AVC), cerebral ischemia; ischemic disturbances in patients with postrupture sub-arachnoid hemorrhage of congenital aneurysms, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease; use as a facilitator of the arterial, cerebral and peripheral blood flow, and a protector of the structural integrity of cell membranes against free radical attacks; use as an aid in the treatment of signs and symptoms of mental deterioration, specially those related to aging; dizziness, chronic headache, labyrinthitis, poor concentration, disorientation, compromising of memory, lack of initiative , mood depression, unsociability, difficulties with daily activities and personal care, emotional lability, reduction in intellectual capacity, behavioral disorders, psychomotor retardation, deficits in learning, senile dementia; neurological deficit in memory, concentration and attention; for the prophylaxis and treatment of symptoms of balance disorders (labyrinthine arteriosclerosis, irritability of the labyrinth, Ménière's syndrome) such as: vertigo, dizziness, buzz, nystagmus, nausea and vomiting, resulting from use as an aid in the treatment of vascular alterations of the inner ear and labyrinth; neurosensorial disorders of vascular origin, in otorhinolaryngology and ophthalmology; and in the treatment of migraine, of subjective symptoms associated with arterial hypertension, for postapoplectic functional consequences.

A number of products comprising varying concentrations of extract of the above plants are commercially available. The use thereof, so far recommended in the art is related to the treatment of physical and mental fatigues, neuromuscular asthenia and weariness.

Studies and research now carried out by the present inventors

show new antioxidant and cerebral vasodilator activities related to products based on the above mentioned extracts as confirmed by the data and tests disclosed herein.

The concentration of the extract of each plant of *Trichilia sp.*, particularly *Trichilia catigua*, *Paullinia cupana*, *Ptychopetalum olacoides* and *Zingiber officinale*, in the product or pharmaceutical composition of the present invention ("Product comprising extract of Catuama") is as follows:

Liquid formulation:		
Component	% (m/v)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	0.50 to 5.50	0.50 to 5.0
Extract of *Paullinia cupana*	0.10 to 7.50	0.1 to 5.0
Extract of *Ptychopetalum olacoides*	0.01 to 5.50	0.01 to 5.0
Extract of *Zingiber officinale*	0.10 to 2.00	0.1 to 0.40
Suitable excipient	79.50 to 99.29	84.60 to 99.24

Solid formulation:		
Component	% (m/m)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	5 to 50	30 to 50
Extract of *Paullinia cupana*	2 to 30	10 to 21
Extract of *Ptychopetalum olacoides*	0.2 to 15.0	5.0 to 12
Extract of *Zingiber officinale*	0.50 to 3.0	0.5 to 1.50
Suitable excipient	2.0 to 92.30	15.5 to 54.5

In its dry and excipient-free form, extract of Catuama comprises:

Formulation		
Component	% (m/m)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	17 to 40.0	22.0 to 34.0
Extract of *Paullinia cupana*	24.0 to 57.0	32.0 to 48.0
Extract of *Ptychopetalum olacoides*	17.0 to 40.0	22.0 to 34.0
Extract of *Zingiber officinale*	2.0 to 5.0	2.5 to 4.0

The product may comprise usual excipients for formulation such as preservatives, colorants, carriers, etc. Adequate excipients are well known by those skilled in the art and do not constitute limiting aspects of the invention.

For the purposes of the present invention, all plants of the genus *Trichilia* were found to be useful, such as, e.g., *T. catigua A. Juss.*, *T. clausseni C. DC.*, *T. casaretti C. DC.*, *T. pallida Swartz.* and *T. elegans A. Juss.* According to a preferred embodiment of the present invention, it was found that, among the genera comprised of species *Trichilia sp.*, *Trichilia catigua* is particularly suitable for the intended purposes. Additionally, the materials extracted from *Trichilia sp.* are preferably fragments of the whole plant, more preferably stalk, which are advantageously used as extract, more preferably they are formulated with pharmaceutically acceptable inert carriers. Formulations of Trichilia sp. useful for the present invention can be administered, e.g., orally in the form of tablets, coated tablets, hard and soft gelatin capsules, solutions, emulsions and suspensions; or rectally, in the form of suppositories. Suitable carriers include, but are not limited to, lactose, starch or derivatives thereof, talc, stearic acid or salts thereof in the case of solid formulations for oral administration. Suitable carriers for soft gelatin capsules include vegetable oils, waxes, fats, semi-solid and liquid polyols. Solutions may be prepared comprising selected carriers such as water, polyols and carbohydrates. In the case of suppositories, suitable carriers comprise natural or hardened oils, waxes, fats and polyols.

In addition to the carriers, the formulations of the Catuama extract according to the present invention may contain preservative agents, solubilizing agents, stabilizers, wetting agents, emulsifiers, sweeteners, coloring agents, flavoring agents, tonicity adjustment substances, buffers, coating agents or anti-oxidants.

However, an effective dosage for administration to humans was found to be in the range from 10 mg to 0.5 g Catuama extract.

In the case of pharmaceutical formulations containing Catuama extract, the intended effects can be effectively obtained using from 0.2 to

50% by weight of said extract, based on the total formulation.

The invention will now be described specifically referring to the applicant's product having the name Catuama®, that is already commercialized in Brazil for the treatment of several chronic diseases such as physical and mental fatigue, neuromuscular asthenia and weariness. The pharmaceutical formulations available allow the product to be administered orally. Another advantage of the product is associated with the lack of any reported undesirable or side effects, even when the product is used for long periods of time.

Together, the results discussed herein show that the Catuama extract referred to in this invention (specially Catauma®) exhibits antioxidant and cerebral vasodilator effects, specially when used regularly and for extended periods of time.

The present invention relates to the Catauma extract effects on pathologies requiring the use of antioxidants and prophylaxis or treatment of cerebral vascular dysfunctions. The Catuama extract of the invention (particularly Catuama®) is particularly used in the treatment of pathologies involving vascular, mainly cerebral, alterations caused by an increase of the oxidative stress and the formation of active species of oxygen. The Catuama extract (specially Catuama®) is comprised of substances acting on the central vascular system, neutralizing free radicals, thus being of great clinical importance. Such effects are a result of the synergistic effect between the diverse substances present in the four extracts making up the product. The synergism is defined as the effect arising from the association of low doses of two or more substances exhibiting the same effect. Such concept is particularly important when taking into account the reduction of undesirable effects of said substances. In the specific case of Catuama extract of the present invention (especially the Catuama®), the four plants comprised therein have pharmacological effects that are potentiated when the extracts are used in combination.

Control of free radicals production is of great interest, since this phenomenon is involved in the development of many diseases. Thus, sub-

stances able to inhibit or delay that situation are greatly useful in clinics, loss of memory, vascular alterations, cerebral ischemia, difficulty in movements, difficulty in learning and concentrating, and for neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, which are pathologies associated, at least in part, with extended exposure to an excess of free radicals. Furthermore, it is known that drugs exhibiting the property of neutralizing free radicals are important in slowing the aging process and its consequences. It can therefore be said that continued use of the phytotherapeutic product Catuama extract (specially Catuama®) is highly applicable, since it shows a great capacity to neutralize free radicals.

Brief Description of the Drawings

Figure 1 shows the concentration-response curve for Catuama extract (10-3000 μg/ml) in porcine basilar artery rings with (E+) and without (E-) endothelium pre-contracted with serotonin (1 μM). Each point represents the average of 4 to 5 experiments and the vertical bars indicate the E.P.M.

Figure 2 shows the effect of Catuama extract (1-100 μg/ml) on nitrite/nitrate levels in peritoneal macrophages of mice stimulated with LPS (1 μg/ml) and INF-γ (10 U/ml). The results are expressed as the average ± E.P.M. The asterisks indicate the significance when compared to the control group (stimulated), $*p < 0.05$ $**p < 0.01$ ($n = 3$).

Figure 3 shows the effect of Catuama extract (1-100 μg/ml) on cell viability of peritoneal macrophages from mice evaluated by means of the MTT method. The results are expressed as the average ± E.P.M. ($n = 3$).

Figure 4 shows the effect of Catuama extract (1-500 μg/ml) on the formation of free radicals from oxidation of deoxyribose. Each result represents the average of 4 to 5 samples and the vertical bars represent the E.P.M.

Figure 5 shows the effect of Catuama extract (10-70 μg/kg) on the formation of superoxide anion radical from the xanthine/xanthine oxidase system. Each group represents the average of 4 to 5 samples and the vertical bars represent the E.P.M.

Figure 6 shows the effect of Catuama extract (10-70 μg/kg) on the formation of superoxide anion radical from the superoxide dismutase enzyme assay. XO (xanthine oxidase), SOD (superoxide dismutase). Each result represents the average of 4 to 5 samples and the vertical bars represent the E.P.M.

The illustrative test examples below are given for a better description of the present invention. However, the data and procedures illustrated therein refer to certain embodiments of the present invention and are not to be construed as limiting the scope thereof.

The following tests were carried out using a composition of extracts in the solid and dry form (herein referred to as Catuama extract) as follows:

Formulation	
Component	% (m/m)
Extract of *Trichilia sp* (specially *Trichilia catigua)*	29.5
Extract of *Paullinia cupana*	37.6
Extract of *Ptychopetalum olacoides*	29.5
Extract of *Zingiber officinale*	3.4

Cerebral Vasodilator Effect

In order to evaluate the relaxing effect of Catuama extract on cerebral vessels, basilar arteries obtained from male and female swine weighing about 100 kg were used. The skulls of the animals were opened and the brain dissected and put into ice-cooled Krebs Henseleit's solution. The basilar arteries were removed and, after cleaning and removing adjacent tissues, cut into rings (2 to 3 mm) which were mounted within glass vats containing 5 ml Krebs Henseleit's solution having the following composition (mM): NaCl 118.0, KCl 4.4, $MgSO_4$ 1.1, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and $C_6H_{12}O_6$ 11.0 (pH 7.2 kept at 37°C and aerated with 95% O_2 and 5% CO_2). The tissues were subjected to 1 g tension and remained in equilibrium for 60 min before the addition of the extracts to the bath (equilibrium period), the nutritive solution being replaced by a fresh one every 20 minutes. Subsequent to the equilibrium period, the preparations were pre-contracted with

serotonin (1 μM) and the endothelium integrity was evaluated by the bradykinin capacity (BK, 1 μM), a known vaso-relaxing peptide, to promote relaxation. Preparations were considered to have the intact endothelium when the BK caused a relaxation greater than 40%. Changes in tension (contraction and relaxation) were isometrically measured by means of force transducers (TRI - 201) and read with polygraph 6006 from Letica Instrumentos Cientificos. Sub-maximum serotonin-induced contractile response was considered to be 100% and all subsequent responses were calculated as a percentage of such value. The results were expressed as percentage values of contraction and relaxation relative to the controls.

After the equilibrium period, said preparations were contracted by the addition of serotonin (1 μM) and after a plateau is reached for the contractile response (approximately 5 minutes), a single cumulative concentration-relaxation response curve was obtained for the Catuama extract (10-3000 μg/ml).

The results obtained from the assays for determining the cerebral vasodilator activity are shown hereinbelow.

The addition of increasing cumulative concentrations of Catuama extract caused concentration-dependent relaxation in the isolated porcine basilar artery having an endothelium showing an EC_{50} 588 (182 - 1095) μg/ml and R_{max} 100 ± 0% (figure 1). When the endothelium was purposely removed, as confirmed by the absence of a relaxing effect to bradykinin, the relaxation caused by Catuama extract was significantly reduced (73 ± 3% inhibition) (figure 1). Considering that the basilar artery is an important vessel for the distribution of arterial blood to the brain, these results explain the use of Catuama extract as a cerebral vasodilator.

A) Effect on the Production of Nitric Oxide

The direct effect of Catuama extract on the production of the main vaso-relaxing and nitric oxide free radicals-producing agent was evaluated in *in vitro* macrophages. These cells were obtained from the peritoneum of 3 month-old male mice intraperitoneally injected with 3% thioglycolate (3 ml). Four days later, the peritoneal cavity was washed with 10 ml of

buffered saline (PBS, composition mmol/l: NaCl 137, KCl 2.7, phosphate buffer 10). The peritoneal wash was centrifuged at 1500 rpm and the precipitate washed with DMEM (nutritive medium). Then, the cells were resuspended in DMEM at a density of 5 x 10^6 cells/ml. The cells were cultured in 96-well plates and incubated for 2 hours at 37°C inside a CO_2 incubator, to allow the adherence of the macrophages. The plate was washed with DMEM (heated at 37°C) for removing non-adhered cells. Subsequently, the medium was replenished and Catuama extract at varying concentrations (1-100 μg/ml) was added. The cells were stimulated with 1 μg/ml LPS and 10 U/ml IFN-δ and incubated for 36 hours. The cells incubated only with LPS and IFN-δ were used as the control for the maximum production of nitric oxide. The incubation time elapsed, and the supernatant was collected for measuring the concentration of nitrite/nitrate (NO^x). First, the conversion of nitrate into nitrite was carried out by incubating the supernatants with bacterium *Escherichia coli* (6 x 10^6 bacteria/ml) for 3 hours. After centrifugation to separate the bacteria, 100 μl of supernatant was added to 100 μl Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine in H_2O). The results were spectrophotometrically determined with a device for ELISA at 550 nm and correlated with a standard curve of sodium nitrate (0-150 μM) which was run in parallel with the assay.

B) Evaluation of Cell Viability

The cell viability of the peritoneal macrophages from the mice was measured through mitochondrial activity-dependent reduction of MTT [3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] to MTT-formazan. The macrophages were incubated in 96-well plates with culture medium and Catuama extract (1-100 μg/ml), for 36 hours at 37°C, in a CO_2 incubator. After this time, the culture medium was removed and the cells were reincubated with MTT (5 mg/ml) for 4 hours at 37°C. Following the incubation period, the MTT-formazan crystals formed were dissolved in isopropyl alcohol containing HCl (0.04 N). The MTT-formazan complex was spectrophotometrically measured at 550 nm. The untreated cells were considered as having a cell viability of 100%.

The results obtained from the assays for determining the effect on the production of nitric oxide are disclosed hereinbelow.

a) Effect on the Production of Nitric Oxide

The presence of Catuama extract has caused a significant inhibition of the production of NO^x at every concentration (1-100 µg/ml) (figure 2). The maximum inhibition produced by the Catuama extract was 93.1 ± 1.03% with a CI_{50} of 15.53 (11.81 - 20.41) µg/ml.

b) Evaluation of Cell Viability

The results obtained with Catuama extract (figure 3) show that incubation of macrophages with the product did not significantly interfere with the cell viability of peritoneal macrophages at any of the concentrations tested. It can therefore be said that at the same concentrations where Catuama extract caused an inhibition of the formation of NO, it did not interfere with the viability of the macrophages, therefore excluding possible toxic effects of the same on the cells .

Evaluation of the Antioxidant Effect

a) Production and Detection of the Hydroxyl Radical ($HO^{\cdot}$)

The procedure used is based on the oxidation of deoxyribose for generating $HO^{\cdot}$ from Fenton's reaction between hydrogen peroxide and Fe (III)-NTA. The chromogen was spectrophotometrically determined at 532 nm. 0.1 mM nitriloacetic acid and 0.025 mM $FeCl_3$ were pre-incubated for 10 minutes. Shortly thereafter, 80 mM KH_2PO_4, 2.8 mM deoxyribose, 1.4 mM H2O2, in addition to the Catuama extract (1-500 µg/ml) or carrier, were added. 20 minutes later, 1 ml of 1% thiobarbituric acid and 2.3% trichloroacetic acid were added, the samples were heated at 100°C (5 minutes), and were then immediately put on ice. The samples were spectrophotometrically (532 nm) analyzed and the results were expressed as a percentage of the oxidation of deoxyribose. For each concentration of the product utilized, a blank was employed for the original color of the sample to be deducted from the result.

b) Production and Detection of Superoxide Anion ($O_2^{\cdot}$)

The superoxide anion radical is generated through the reaction

catalyzed by the xanthine/xanthine oxidase system. Based on this principle, the possibility that Catuama extract might act on the xanthine oxidase activity, impeding the formation of the O_2^- anion was first verified. Xanthine oxidase activity was evaluated by the spetrophotometric (295 nm) measurement of the formation of uric acid derived from xanthine in the presence or absence of Catuama extract. Since no changes in the xanthine oxidase enzyme action were observed, the next step was to verify the product capacity for sequestering O_2^- radicals. The formation of this free radical was verified in the presence and absence of Catuama extract (10-70 μg/ml) or carrier. The reactions were carried out in a medium containing 100 μM xanthine, 0.1 M phosphate buffer, pH 7.8, 600 μM NBT and 0.04 U/ml xanthine oxidase (incubated at 25°C for 10 minutes). The O_2^- radical formed was spectrophotometrically monitored at 560 nm for the reduction of nitrobluetetrazolium (NBT).

c) Enzymatic Antioxidant Activity

The superoxide dismutase assay (SOD) was carried out in order to verify the Catuama extract activity on glutathione S-transferase enzyme. This enzyme has the capacity to transform O_2^- radicals, thus being a naturally occurring antioxidant. The O_2^- radical is generated by the reaction catalyzed with the xanthine/xanthine oxidase system and its formation was monitored by the reduction of NBT, as previously described. The reactions were carried out in a medium containing 100 μM xanthine, 0.1 M phosphate buffer, pH 7.8, 600 μM NBT and 0.04 U/ml xanthine oxidase, and 100 U/ml SOD (incubated at 25°C for 10 minutes), aiming to verify that enzyme capacity for sequestering O_2^- radicals produced by the reduction of NBT. The SOD activity was evaluated by spectrophotometrically (560 nm) measuring the percentage reduction of NBT. The enzymatic antioxidant activity was evaluated in the presence and absence of Catuama extract (30-60 μg/ml) or carrier.

The results from the assays for determining the antioxidant action are disclosed hereinbelow.

a) Production and Detection of the Hydroxyl Radical (HO^-)

Catuama extract was shown to be very effective in impeding the

oxidation of deoxyribose (figure 4). The product caused 61% inhibition with a CI_{50} of 13.21 μg/ml, it being possible to reach the maximum limit for this assay due to the high speed in which the reaction takes place.

b) Production and Detection of Superoxide Anion (O_2^-)

Catuama extract (10-70 μg/ml) caused a significant decrease in the formation of xanthine by xanthine oxidase, indirectly suggesting a decrease in the production of superoxide anion radical (figure 5). This product inhibited about 63% exhibiting a CI_{50} of 2.3 μg/ml. Knowing that Catuama extract does not interfere with the enzyme activity, it can be said that the product acts as a sequesterant of O_2^- radical.

c) Enzymatic Antioxidant Activity

Catuama extract did not change the enzyme activity in the assay (figure 6), with just a decrease in the quantity of superoxide anion radical produced by the xanthine/xanthine oxidase system occurring, indicating the antioxidant activity thereof is due only to the sequesteration of O_2^- radicals (figures 6 and 7). These results together suggest an important action of Catuama extract as an antioxidant.

CLAIMS

1. Use of a product comprising Catuama extract, comprising the species *Trichilia sp.*, *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*), wherein said product is an antioxidant or cerebral vasodilator agent.

2. The use of claim 1, wherein the composition of said product is as follows

Liquid formulation:		
Component	% (m/v)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	0.50 to 5.50	0.50 to 5.0
Extract of *Paullinia cupana*	0.10 to 7.50	0.1 to 5.0
Extract of *Ptychopetalum olacoides*	0.01 to 5.50	0.01 to 5.0
Extract of *Zingiber officinale*	0.10 to 2.00	0.1 to 0.40
Suitable excipient	79.50 to 99.29	84.60 to 99.24

Solid formulation:		
Component	% (m/m)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	5 to 50	30 to 50
Extract of *Paullinia cupana*	2 to 30	10 to 21
Extract of *Ptychopetalum olacoides*	0.2 to 15.0	5.0 to 12
Extract of *Zingiber officinale*	0.50 to 3.0	0.5 to 1.50
Suitable excipient	2.0 to 92.30	15.5 to 54.5

or in its dry and excipient-free form, comprises:

Formulation		
Component	% (m/m)	
	Generic	**Preferred**
Extract of *Trichilia sp* (specially *Trichilia catigua)*	17 to 40.0	22.0 to 34.0
Extract of *Paullinia cupana*	24.0 to 57.0	32.0 to 48.0
Extract of *Ptychopetalum olacoides*	17.0 to 40.0	22.0 to 34.0
Extract of *Zingiber officinale*	2.0 to 5.0	2.5 to 4.0

3. The use of any of the preceding claims, wherein said product is Catuama®.

4. The use of any of the preceding claims, for the prophylaxis and treatment of cerebral vascular dysfunctions, arteriosclerosis, stroke (AVC), cerebral ischemia; ischemic disturbances in patients with postrupture subarachnoid hemorrhage of congenital aneurysms, neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease.

5. The use of any of the preceding claims, wherein said product acts as a facilitator of the arterial, cerebral and peripheral blood flow, and a protector of the structural integrity of cell membranes against free radical attacks.

6. The use of any of the preceding claims, wherein it is an aid in the treatment of signs and symptoms of mental deterioration, specially those related to aging: dizziness, chronic headache, labyrinthitis, poor concentration, disorientation, compromising of memory, lack of initiative, mood depression, unsociability, difficulties with daily activities and personal care emotional lability, reduction in intellectual capacity, behavior disorders, psychomotor retardation, deficit in learning, senile dementia; neurological deficit in memory, concentration and attention.

7. The use of any of the preceding claims, for the prophylaxis and treatment of symptoms of balance disorders (labyrinthine arteriosclerosis, irritability of the labyrinth, Ménière's syndrome) such as: vertigo, dizziness, buzz, nystagmus, nausea and vomiting.

8. The use of any of the preceding claims, wherein said product is used as an aid in the treatment of vascular alterations of the inner ear and labyrinth.

9. The use of any of the preceding claims, wherein it is used as an aid in the treatment of neurosensorial disorders of vascular origin, in otorhinolaryngology and ophthalmology.

10. The use of any of the preceding claims, for the treatment of migraine, vertigo and nausea.

11. The use of any of the preceding claims, for the treatment of

subjective symptoms associated to arterial hypertension.

12. The use of any of the preceding claims, for postapoplectic functional consequences.

13. A pharmaceutical composition comprising a product of plant extracts comprising the species *Trichilia catigua* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*) for the treatment and/or prevention of any disorders requiring the use of an antioxidant or cerebral vasodilator agent.

14. The pharmaceutical composition of claim 13, wherein the Catuama extract has a composition as defined in claim 2.

15. The pharmaceutical composition of any of claims 12, 13 or 14, wherein said product is Catuama®.

16. The pharmaceutical composition of any of the preceding claims, wherein said pharmaceutical composition is used for the treatment and/or prevention of any disorders as described in claims 2 to 12.

17. A method for treating and/preventing cerebral vascular dysfunctions and disorders caused by the inadequate presence of free radicals, comprising administering a product of Catuama extract comprising the species *Trichilia sp.* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*) to a patient in need thereof.

18. The method of treatment and/or prevention of claim 17, wherein said product has a composition as defined in claim 2.

19. The method of treatment and/or prevention of any of the preceding claims, wherein said product is Catuama®.

20. The method of treatment and/or prevention of any of the preceding claims, wherein said method is used for the treatment and/or prevention of any disorders as described in claims 2 to 12.

21. Use of a product comprising Catuama extract, comprising the species *Trichilia sp.* (*Meliaceae*), *Paullinia cupana* (*Sapindaceae*), *Ptychopetalum olacoides* (*Olacaceae*) and *Zingiber officinale* (*Zingiberaceae*), wherein said product is used for the treatment and/or prevention of cerebral

vascular dysfunctions and disorders caused by the inadequate presence of free radicals.

22. The use of claim 21, wherein said product has the composition as defined in claim 2.

23. The use of any of claims 21 or 22, wherein said product is Catuama®.

24. The use of any of claims 21, 22 or 23, wherein said product is used for the treatment and/or prevention of any disorders as described in claims 2 to 12.

Fig. 1

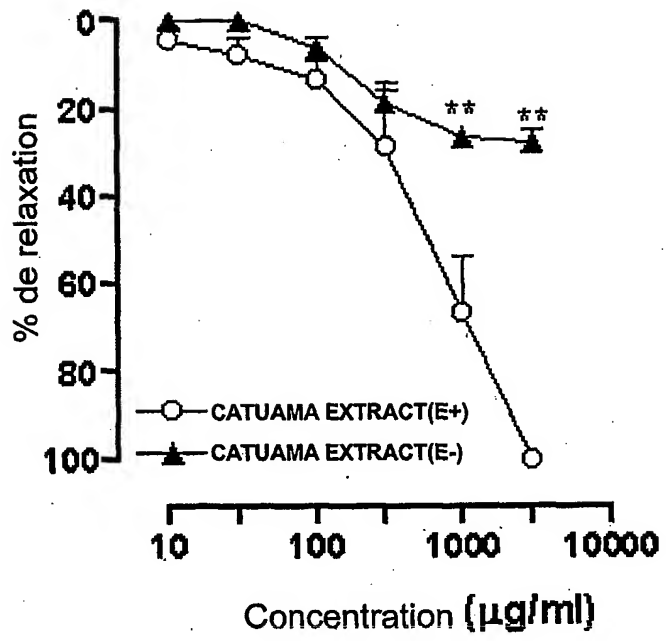

0
20
40
60
80
100
% de relaxation
**
**
CATUAMA EXTRACT(E+)
CATUAMA EXTRACT(E-)
10
100
1000
10000
Concentration (µg/ml)

Fig. 2

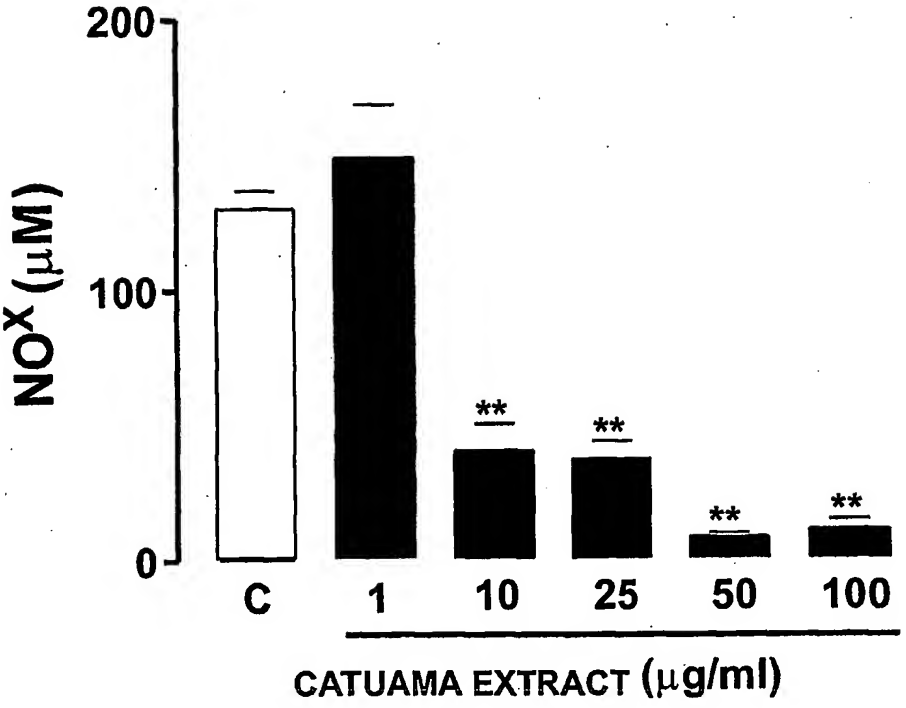


Fig. 3

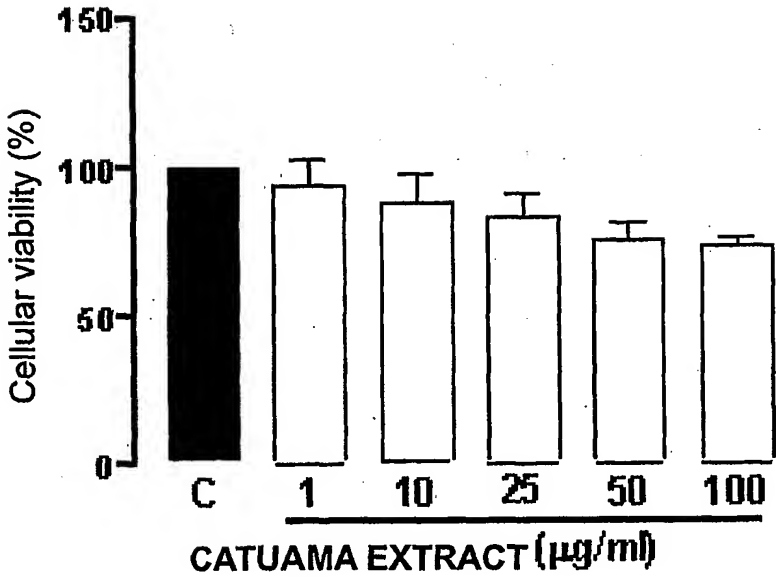

150
100
50
0
Cellular viability (%)
C
1
10
25
50
100
CATUAMA EXTRACT (µg/ml)

Fig. 4

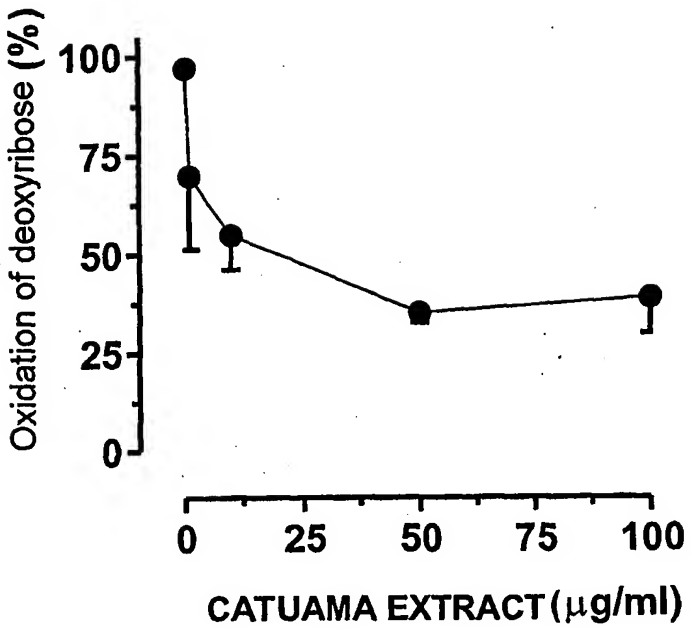

Oxidation of deoxyribose (%)
100
75
50
25
0
0
25
50
75
100
CATUAMA EXTRACT (μg/ml)

Fig. 5

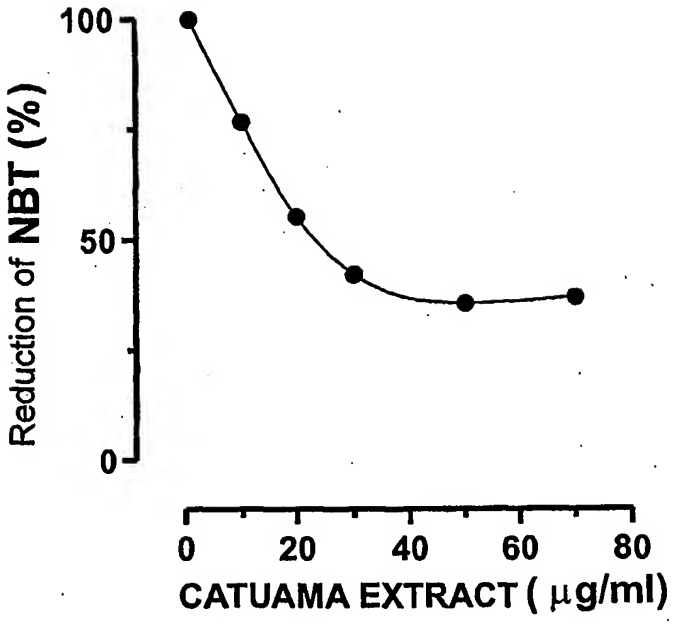
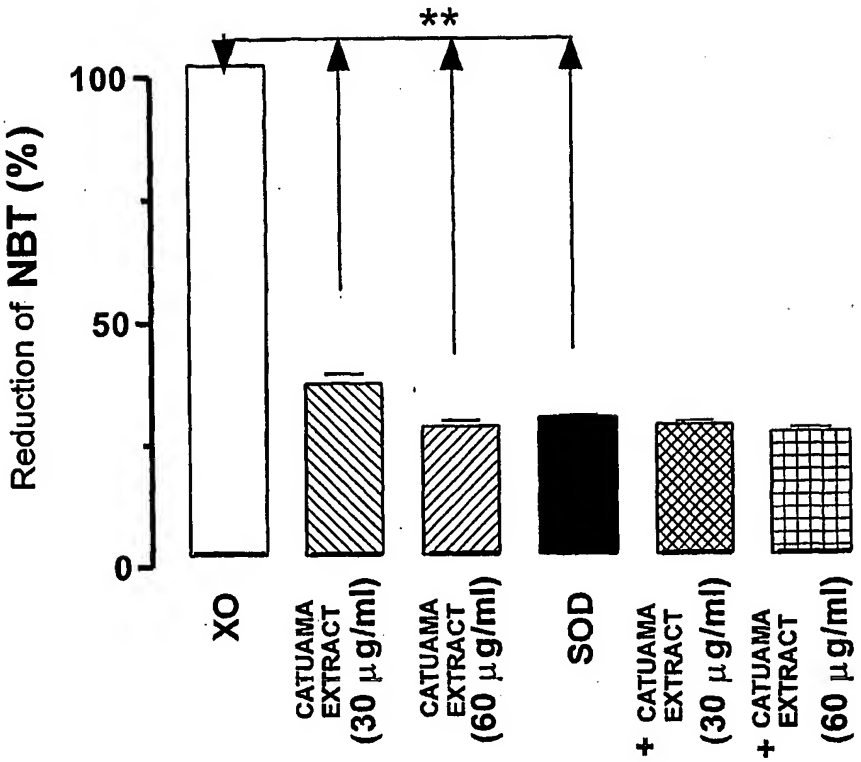


Fig. 6


**
100
50
0
Reduction of NBT (%)
XO
CATUAMA EXTRACT (30 µg/ml)
CATUAMA EXTRACT (60 µg/ml)
SOD
+ CATUAMA EXTRACT (30 µg/ml)
+ CATUAMA EXTRACT (60 µg/ml)

INTERNATIONAL SEARCH REPORT

I onal Application No	PCT/BR 01/00091

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/78 A61P7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, FSTA, MEDLINE, PASCAL, LIFESCIENCES, CHEM ABS Data, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CALIXTO J B ET AL: "HERBAL MEDICINE CATUAMA INDUCES ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASORELAXANT ACTION ON ISOLATED VESSELS FROM RATS, GUINEA-PIGS AND RABBITS" PHYTOTHERAPY RESEARCH, JOHN WILEY & SONS LTD. CHICHESTER, GB, vol. 11, no. 1, 1 February 1997 (1997-02-01), pages 32-38, XP002061880 ISSN: 0951-418X the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 2002	13/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Rempp, G

Form PCT/ISA/210 (second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 4-12,17-24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Claims Nos.: 4-12,17-24

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

inal Application No
PCT/BR 01/00091

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	VAZ Z R ET AL: "ANALGESIC EFFECT OF THE HERBAL MEDICINE CATUAMA IN THERMAL AND CHEMICAL MODELS OF NOCICEPTION IN MICE" PHYTOTHERAPY RESEARCH, JOHN WILEY & SONS LTD. CHICHESTER, GB, vol. 11, no. 2, 1 March 1997 (1997-03-01), pages 101-106, XP002061879 ISSN: 0951-418X	
A	WO 99 02172 A (CATARINENSE S A LAB ;MIKIO KASSUYA ROBERTO (BR); MOREIRA EDUARDO A) 21 January 1999 (1999-01-21)	

INTERNATIONAL SEARCH REPORT

formation on patent family members

In ɔnal Application No
PCT/BR 01/00091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902172 A	21-01-1999	BR 9703946 A	09-03-1999
		AU 3843697 A	08-02-1999
		WO 9902172 A1	21-01-1999
		JP 2001509486 T	24-07-2001
		US 6335039 B1	01-01-2002

L4: Entry 27 of 29 File: DWPI Dec 9, 2002

DERWENT-ACC-NO: 2003-140416
DERWENT-WEEK: 200452

TITLE: Use of a product containing Catuama extract comprising Trichilia species, sapindaceae, olacaceae and zingiberaceae plant extracts as an antioxidant or cerebral vasodilator agent for the treatment of, e.g. stroke

INVENTOR: ANDRE, E ; BATISTA CALIXTO, J ; BEIRITH, A ; CARINI, D D A ; FUJII, T ; SCHLEMPER, V ; SILVA FILHO, O ; SOARES FERNANDES, E ; DE ALMEIDA CABRINI, D ; SILVA, O ; DE ALMEIDA CARINI, D

PATENT-ASSIGNEE:

ASSIGNEE	CODE
LAB CATARINENSE SA	CATAN

PRIORITY-DATA: 2001BR-0002185 (May 30, 2001)

Search Selected Search ALL Clear

PATENT-FAMILY:

	PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
☐	AU 2001297991 A1	December 9, 2002		000	A61K035/78
☐	WO 200296441 A1	December 5, 2002	E	015	A61K035/78
☐	BR 200102185 A	February 18, 2003		000	A61K035/78

DESIGNATED-STATES: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
AU2001297991A1	July 31, 2001	2001AU-0297991	
AU2001297991A1		WO 200296441	Based on
WO 200296441A1	July 31, 2001	2001WO-BR00091	
BR 200102185A	May 30, 2001	2001BR-0002185	

INT-CL (IPC): A61K 35/78; A61P 7/00; A61P 9/08

ABSTRACTED-PUB-NO: WO 200296441A

BASIC-ABSTRACT:

NOVELTY - A product containing Catuama extract comprising the species Trichilia sp. (a), Paullinia cupana (sapindaceae) (b), Ptychopetalum olacoides (olacaceae) (c) and Zingiber officinale (zingiberaceae) (d) is used as an antioxidant or cerebral vasodilator agent.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a pharmaceutical composition comprising a product of plant extracts comprising the species Trichilia catigua (meliaceae), (b), (c) and (d) for the treatment and/or prevention of any disorders requiring the use of an antioxidant or cerebral vasodilator agent.

ACTIVITY - Antiarteriosclerotic; Cerebroprotective; Nootropic; Neuroprotective; Antiparkinsonian; Antidepressant; Antiemetic; Antimigraine; Hypotensive.

In order to evaluate the relaxing effect of Catuama extract on cerebral vessels, basilar arteries obtained from male and female swine (100 kg) were used. Subsequent to the equilibrium period, the preparations were pre-coated with serotonin (1 mu M). After the equilibrium period, the preparation was contracted by the addition of serotonin (1 mu M).

The addition of increasing cumulative concentrations of catuama extract showed an EC50 588 (182 - 1095) mu g/ml. When the endothelium was purposely removed, the relaxation caused by catuama extract was significantly reduced to 73 plus or minus 3 % inhibition.

MECHANISM OF ACTION - None given.

USE - The product is used:

(1) as an antioxidant or cerebral vasodilator agent for the treatment of cerebral vascular dysfunction, arteriosclerosis, stroke, cerebral ischemia, ischemic disturbances in patients with postrupture subarachnoid hemorrhage of congenital aneurysms or neurodegeneration diseases such as Parkinson's disease and Alzheimer's disease;

(2) as a facilitator of the arterial, cerebral and peripheral blood flow, and a protector of the structural integrity of cell membranes against free radical attacks;

(3) in the treatment of signs and symptoms of mental deterioration, aging, dizziness, chronic headache, labyrinthitis, poor concentration, disorientation, compromising of memory, lack of initiative, mood depression, unsociability, difficulties with daily activities and personal care emotional lability, reduction in intellectual capacity, behavior disorders, psychomotor retardation, deficit in learning, senile dementia or neurological deficit in memory, concentration and attention;

(4) balance disorder (labyrinthine arteriosclerosis, irritability of the labyrinth, Meniere's syndrome), vertigo, buzz, nystagmus, nausea, and vomiting;

(5) in the treatment of vascular alteration of the inner ear and labyrinth, neurosensorial disorders of vascular origin, in otorhinolaryngology, and ophthalmology, migraine, subjective symptoms of associated to arterial hypertension, for postapoplectic functional consequences or disorders caused by inadequate presence of free radicals (all claimed).

ADVANTAGE - The product shows a great capacity to neutralize free radicals.

CHOSEN-DRAWING: Dwg.0/6

TITLE-TERMS: PRODUCT CONTAIN EXTRACT COMPRISE SPECIES PLANT EXTRACT ANTIOXIDANT CEREBRAL VASODILATING AGENT TREAT STROKE

DERWENT-CLASS: B04

CPI-CODES: B04-A08C2; B04-A09; B04-A10; B14-E05; B14-F02C; B14-F02D; B14-F07; B14-J01A1; B14-J01A3; B14-J01A4; B14-N02; B14-N16;

CHEMICAL-CODES:

Chemical Indexing M1 *01*
Fragmentation Code
M417 M423 M781 M905 P443 P444 P446 P450 P451 P510
P520 P528 P625 P814 P921
Specfic Compounds
A00GTK A00GTT A00GTU

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C2003-035631

Previous Doc Next Doc Go to Doc#